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T-cadherin expression alternates with migrating neural crest cells in the trunk of the avian embryo

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Summary

Trunk neural crest cells and motor axons move in a segmental fashion through the rostral (anterior) half of each somitic sclerotome, avoiding the caudal (posterior) half. This metameric migration pattern is thought to be caused by molecular differences between the rostral and caudal portions of the somite. Here, we describe the distribution of T-cadherin (truncated-cadherin) during trunk neural crest cell migration. T-cadherin, a novel member of the cadherin family of cell adhesion molecules was selectively expressed in the caudal half of each sclerotome at all times examined. T-cadherin immunostaining appeared graded along the rostrocaudal axis, with increasing levels of reactivity in the caudal halves of progressively more mature (rostral) somites. The earliest T-cadherin expression was detected in a small population of cells in the caudal portion of the somite three segments rostral to last-formed somite. This initial T-cadherin expression was observed concomitant with the invasion of the first neural crest cells into the rostral portion of the same somite in stage 16 embryos. When neural crest cells were ablated surgically prior to their emigration from the neural tube, the pattern of T-cadherin immunoreactivity was unchanged compared to unoperated embryos, suggesting that the metameric T-cadherin distribution occurs independent of neural crest cell signals. This expression pattern is consistent with the possibility that T-cadherin plays a role in influencing the pattern of neural crest cell migration and in maintaining somite polarity.

Key words: trunk neural crest cell migration, cell adhesion molecules, chick embryos, somite, sclerotome.

Introduction

Neural crest cells initiate their migration from the neural tube shortly after fusion of the neural folds and neural tube closure. These cells migrate along several well-defined pathways and give rise to numerous derivatives, including pigment cells, Schwann cells, adrenal chromaffin cells and cartilagenous elements of the face (ref. LeDouarin, 1982). In addition, neural crest cells contribute the precursors for most peripheral neurons and glia. In the trunk region, the neural crest-derived sensory and sympathetic ganglia with their associated nerve roots are organized in a segmental pattern along the vertebral column. This segmental organization results from the metameric migratory pattern of neural crest cells that selectively move through the rostral (anterior) half of each somitic sclerotome during early phases in development (Rickmann *et al.* 1985; Bronner-Fraser, 1986; Teillet *et al.* 1987; Lallier and Bronner-Fraser, 1988).

The majority of trunk neural crest cells migrate in a ventral direction away from the neural tube and through the adjacent somites. As the epithelial somites undergo a transition to form the dermatome (presumptive dermis), myotome (presumptive muscle), and sclerotome (presumptive vertebrae), most ventrally migrating neural crest cells invade the rostral half of each sclerotome (see Fig. 1); in contrast, the caudal (posterior) half of each sclerotome lacks migrating neural crest cells (Rickmann *et al.* 1985; Bronner-Fraser, 1986; Teillet *et al.* 1987; Serbedzija *et al.* 1989). Similarly, motor axons, emerging from the ventral neural tube several hours after neural crest cells, extend their growth cones through the rostral half of the somitic sclerotome (Keynes and Stern, 1984). For both populations, the signals responsible for the metameric pattern appear to reside within the somite itself. After experimental rotation of the segmental plate to reverse its rostrocaudal polarity, neural crest cells and motor axons recognize the appropriate rostral portion of the somite even when presented in a caudal position (Keynes and Stern, 1984; Bronner-Fraser and Stern, 1991). Furthermore, in the absence of the somitic sclerotome, neural crest cells and motor axons migrate in a non-segmental pattern (Lewis *et al.* 1981; Tosney, 1988; Stern *et al.* 1986; Kalcheim and Teillet, 1989).

Molecular differences between the cells of the rostral and caudal halves of the somitic sclerotome are likely to account for the selective migration pattern of neural crest cells and motor axons through the rostral half of the somites. Cells in the rostral half of each somite may selectively expose or secrete molecules that attract neural crest cells and/or axons, whereas cells in the caudal half of each somite may contain molecules that are repulsive or inhibitory to cell and/or axon movements. Furthermore, it may be a combination of both attractant molecules in the rostral and repulsive molecules in the caudal half of each sclerotome that regulate the metameric pattern of neural crest cell and/or motor axon locomotion through somite tissues. Some molecular differences between rostral and caudal somites have been noted. Peanut lectin binding glycoproteins of 48 and 55×10^3 M_r have been localized specifically to the caudal half of the somitic sclerotome (Stern *et al.* 1986; Davies *et al.* 1990). In the rostral half of the sclerotome, a 70×10^3 M_r protein (Tanaka *et al.* 1989), butyrylcholinesterase activity (Layer *et al.* 1988), and cytotactin/tenascin/J1 (Tan *et al.* 1987; Mackie *et al.* 1988; Stern *et al.* 1989) have been detected. In addition, differences in the polypeptide composition between rostral and caudal somite portions have been revealed by two-dimensional gel electrophoresis (Norris *et al.* 1989).

Cadherins are a class of cell adhesion molecules that have been suggested to regulate morphogenesis by a calcium-dependent adhesion mechanism (Takeichi, 1988). A novel member of this family, T-cadherin (truncated-cadherin), was recently identified by molecular cloning and sequencing of corresponding cDNAs (Ranscht and Dours, 1989; Ranscht and Dours-Zimmerman, 1991). In the present study, we have examined the onset and distribution of T-cadherin in relation to neural crest cell migration. T-cadherin was localized selectively in the caudal half of the somites at both initial and advanced stages of neural crest cell migration. Furthermore, T-cadherin expression in the caudal half of each somite coincided with the initial penetration of neural crest cells into the rostral half of each somite. This distribution is consistent with a possible role for T-cadherin in influencing the pattern of trunk neural crest cell migration.

Materials and methods

Embryos

White Leghorn chick embryos ranging from stages 11 to 19 (according to the criteria of Hamburger and Hamilton, 1951) were used for this study. Eggs were incubated in a forced air incubator at 37°C until they reached the desired stage of development.

Fixation and tissue processing

Cryostat sections

Embryos were removed from the eggs and washed in Howard's Ringers solution. The embryos were straightened in wax dishes by placing insect pins or cactus needles into their surrounding membranes. They were then fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS) at 4°C overnight, washed in PBS, transferred to 5% sucrose in PBS with azide for 4–24 h, and immersed in 15% sucrose in PBS with azide overnight at 4°C. Embryos were placed in 7% gelatin (Sigma; 300 Bloom) in 15% sucrose/PBS for 3 h at 37°C, and embedded in fresh gelatin. Storage of the embryos was up to two weeks in the refrigerator until the time of sectioning when they were rapidly frozen in liquid nitrogen. 14 μm sections were cut on a Reichardt cryostat and mounted on gelatin-subbed slides.

Paraffin sections

Embryos were fixed in Zenkers fixative for 1.25 h, rinsed in running water for 15 min, and placed in 70% ethanol. Embryos were dehydrated through a series of alcohols, followed by three changes of histosol, and three changes of paraffin before embedding in fresh paraffin. Sections were cut on a Leitz microtome at a thickness of 5 μm.

Immunocytochemistry

Cryostat sections were air dried for 15 min prior to application of antibody. A polyclonal antiserum against T-cadherin (Ranscht and Dours-Zimmermann, 1991) was applied diluted 1:150 in PBS containing 0.1% BSA and 0.05% Triton X-100 at room temperature for two hours or overnight. Sections were washed in phosphate buffer and incubated for one hour with FITC-goat anti-rabbit IgG (Zymed) diluted 1:30 in 0.1 M PBS, pH 7.4, with 1% BSA (PBS/BSA). Slides treated with preimmune instead of immune antiserum gave no immunoreactivity. In some experiments, sections were labelled simultaneously with anti-T-cadherin and the HNK-1 antibody which recognizes migrating neural crest cells (Tucker *et al.* 1984). The HNK-1 antibody is a mouse IgM and was detected using an RITC-rabbit anti-mouse IgM (Axell) for 1 h.

Paraffin sections were rehydrated and stained with the HNK-1 antibody as described above and elsewhere (Bronner-Fraser, 1986). After antibody staining, the cell nuclei in the same sections were stained by placing them in 0.2 $\mu g\,ml^{-1}$ of 4-6-diamidino-2-phenylindole (DAPI) solution in 0.1 M phosphate buffer for 3 min.

Neural crest ablations

Chicken eggs were incubated until they reached stages 11–13 (according to the criteria of Hamburger and Hamilton, 1951). A small hole was made at one end of the egg and 1 ml of albumin was removed to lower the embryo away from the shell. A hole slightly larger than the blastoderm was cut in the shell overlying the embryo. India Ink (Pelikan Fount; diluted 1:4 in Eagle's Minimum Essential Medium containing 15% horse serum and 10% 11-day chick embryo extract) was injected underneath the blastoderm to aid in visualizing the embryo. The vitelline membrane was removed with an electrolytically sharpened tungsten needle.

In order to remove the presumptive neural crest cells, the dorsal portion of the neural tube was excised using glass needles and Dumont no. 5 forceps. The rostral limit of the ablation was typically three to four somites above the most recently formed somite. A length of dorsal neural tube corresponding to six or more somite lengths was removed. Following the operation, embryos were sealed with adhesive tape and returned to the incubator for 24 h prior to fixation. Embryos were prepared for cryostat sectioning and immunocytochemistry as described above.

Analysis of cell density

Longitudinal paraffin sections of 5 μm thickness were stained

with HNK-1 antibody and DAPI as described above. The numbers of somites were counted using the most recently formed somite as a landmark. The sections were viewed through a SIT camera onto a video screen. On randomly selected sections, somite borders were traced and the cell nuclei in a given somite were counted. The line demarcating the rostral and caudal halves of the sclerotome was determined by the distribution of neural crest cells, which selectively migrate through the rostral half only. To determine the number of sclerotomal cells, the number of HNK-1-positive cells was substracted from the total number of cells in the rostral half of the sclerotome. A Student's two-sided *t*-test was used to determine the statistical significance of differences in the number of cells per unit area.

Immunoblotting techniques

Somites were dissected from stage 16–17 chick embryos at levels between the 8th rostral- and 6th caudal-most somites. The tissue was homogenized immediately in cold 10 mM Tris–HCl, pH 7.6, 2 mM $CaCl_2$, 2% Nonidet P40, 1 mM dithiothreiol, 1 mM phenylmethylsulfonyl fluoride, 50 μM leupeptin, 5 μM pepstatin and 4 ng ml^{-1} aprotinin. Proteins were separated by SDS–PAGE (Laemmli, 1970) and transferred to polyvinylidene difluoride transfer membranes (Immobilon P, Millipore) for several hours or overnight (Towbin *et al.* 1979). Non-specific binding was blocked with 5% nonfat dry milk in TBST (10 mM Tris–HCl, pH 8.0, 150 mM NaCl, 0.05% Tween 20) for 1 h. T-cadherin was probed with rabbit anti-T-cadherin antiserum (1:500, Ranscht and Dours-Zimmermann, 1991) followed by ^{125}I-goat anti-rabbit immunoglobulin (1×10^6 cts min^{-1} ml^{-1}) diluted in TBST. Each incubation was for one hour and followed by five washes in TBST. Reacted blots were exposed for 1–5 h to Kodak XAR5-film with an intensifying screen.

Results

Neural crest cells begin to emigrate from the neural tube shortly after neural tube closure. The majority of neural crest cells move ventrally through the rostral half of each somite (Fig. 1), first at rostral axial levels of the embryo and subsequently at more caudal levels. Therefore, several stages of neural crest cell migration exist simultaneously within a single embryo. Neural crest migration is well advanced in the rostral (older) portion of the embryo, when it is just beginning in more caudal (younger) regions. We have performed most of our analyses on stage 16–18 chick embryos because early and advanced stages of neural crest cell migration exist simultaneously in the embryo at these times.

Anti-T-cadherin antiserum primarily detects a 90×10^3 M_r protein in somites

Since T-cadherin is expressed in a variety of neuronal and non-neuronal tissues (Ranscht and Dours-Zimmermann, 1991), immunoblots were used to determine if the antiserum specifically identifies T-cadherin in somite tissue. Somites dissected from stage 16–17 chicken embryos were homogenized in the presence of Ca^{2+} and protease inhibitors, separated by SDS–PAGE and transferred to polyvinylidene difluoride membranes. After reaction with anti-T-cadherin antiserum and ^{125}I-goat anti-rabbit IgG, a single polypeptide of approximately 90×10^3 M_r was detected (Fig. 2). This reactivity compares well with results reported elsewhere (Ranscht and Dours-Zimmerman, 1991) and indicates that the immunoreactivity described below reflects genuine T-cadherin.

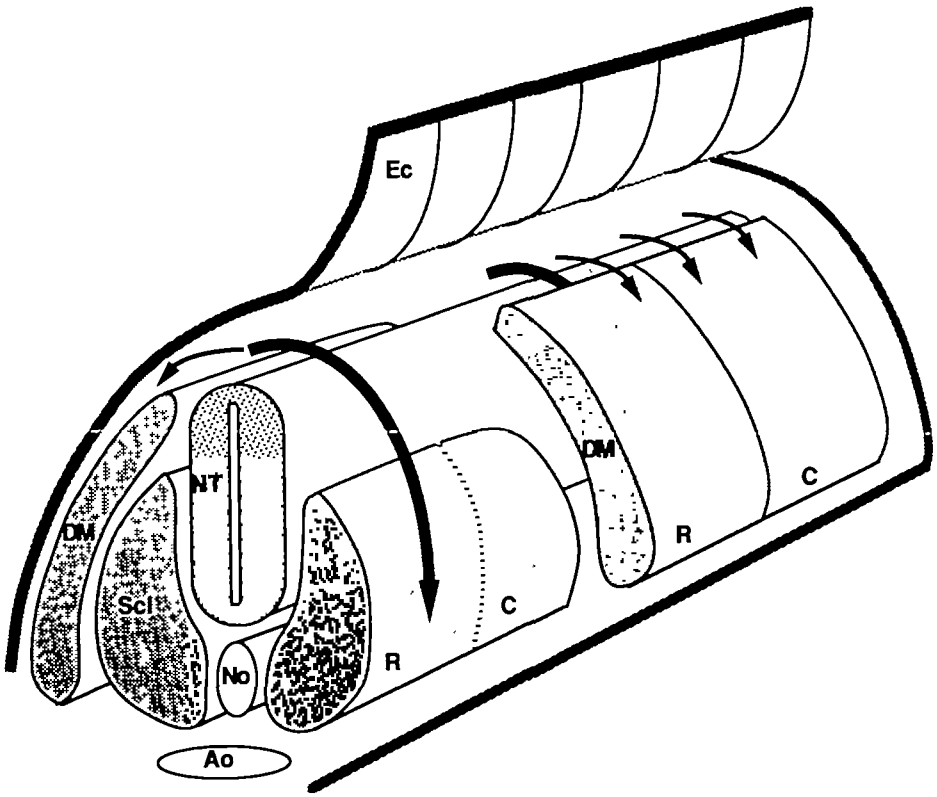


Fig. 1. Schematic diagram illustrating the early pathways of trunk neural crest cell migration. Neural crest cells emerge from the neural tube (NT) and proceed either ventrally (indicated by large curved arrow) through the somitic sclerotome (Scl) or dorsolaterally (indicated by small curved arrow) between the ectoderm (Ec) and dermomyotome (DM). The ventrally migrating cells only move through the rostral (R) half of each sclerotome and are not observed in the caudal (C) half (Rickmann *et al.* 1985; Bronner-Fraser, 1986). Neural crest cells also avoid the region around the notochord (No) (Pettway *et al.* 1990) Ao=dorsal aorta.

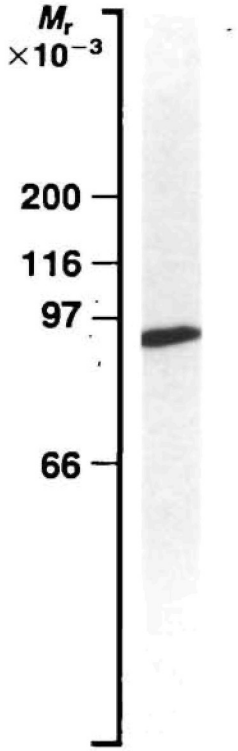


Fig. 2. Reactivity of T-cadherin antiserum on immunoblots. In somites isolated from stage 16–17 chicken embryos, the anti-T-cadherin antiserum used in this study recognizes a single polypeptide band of approximately 90×10^3 M_r.

Appearance of T-cadherin in stage 16–18 chick embryos

T-cadherin immunoreactivity within somite tissue was observed in the caudal half of the sclerotome at all times examined. A gradation of staining intensity was observed along the rostrocaudal axis with most intense immunoreactivity at rostral levels (Fig. 3A,D). Using the last-formed somites as reference, the caudal-most region containing detectable T-cadherin immunoreactivity was about three somites rostral to the last-formed somite; T-cadherin appeared on a small population of cells in the caudal portion of this somite (Fig. 3C). The level of immunoreactivity increased with distance from the last somite, with rostral portions of the embryo expressing higher levels of T-cadherin than caudal ones (Fig. 3A,D).

As the somites completed the epithelial–mesenchymal transition to form the dermomyotome and sclerotome, T-cadherin expression became restricted to the sclerotome. In newly formed sclerotomes, T-cadherin immunoreactivity appeared as a diffuse signal. Although the immunoreactivity was most prominent in the caudal half of the somite, there was no sharp discontinuity in staining intensity distinguishing the rostral from caudal halves of the sclerotome. Rather, the transition between the caudal and rostral halves was diffuse and gradual. Approximately six somites rostral to the last formed somite, the level of immunoreactivity in the caudal somite region intensified markedly. In addition to the prominent immunoreactivity in the caudal half of the somite, a few faint fibrils of T-cadherin immunoreactive material appeared to extend into rostral portions of the sclerotome.

Eleven to twelve somites rostral to the last formed somite, both the morphology of the somites and the T-cadherin immunoreactivity underwent an apparent transition. T-cadherin expression appeared more distinct, with a sharp discontinuity in immunostaining separating the caudal from the rostral halves of the sclerotome. At this stage and beyond, T-cadherin was clearly associated with the surfaces of caudal sclerotomal cells (Fig. 4), and absent from the rostral sclerotome cells. Morphologically, the somites appeared more compact and shorter in rostral–caudal extent than somites in less mature regions of the embryo.

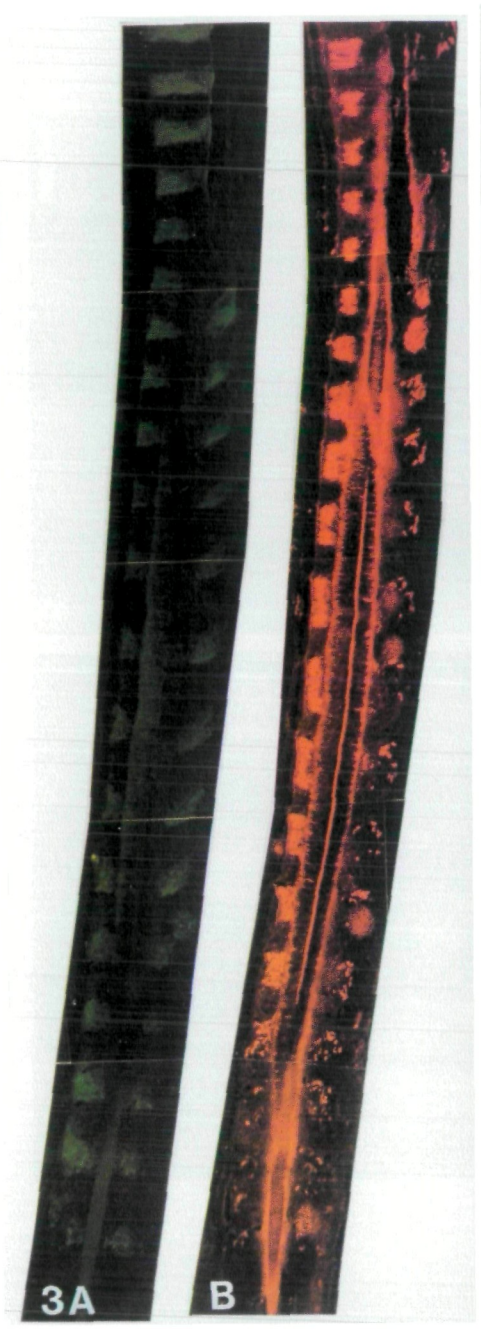
In transverse sections at the level of the wingbud, T-cadherin immunoreactivity in the sclerotome appeared on the surface of all cells in the caudal half of the sclerotome including the dorsal portion of the sclerotome, the region between the dermomyotome and neural tube, and the ventral portion of the sclerotome surrounding the notochord (Fig. 5A). In contrast, immunoreactivity was absent from all regions of the rostral half of the sclerotome. In more caudal (younger) regions of the embryo, T-cadherin immunoreactivity was observed in the dorsal sclerotome, but appeared to be absent from the ventral sclerotome surrounding the notochord (Fig. 5B). The dermomyotome and subepidermal region appeared to lack T-cadherin immunoreactivity at all times examined. Migrating neural crest cells were not seen to express T-cadherin, though motor axons that also move through the rostral half of the sclerotome, expressed T-cadherin at all stages examined.

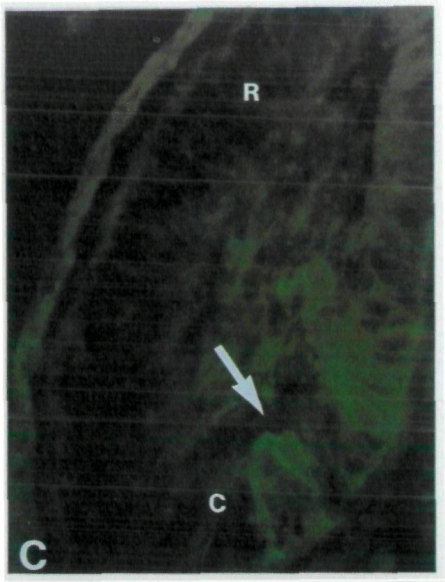
T-cadherin expression in stage 16–18 embryos inversely correlates with neural crest cell migration

The first neural crest cells to emigrate from the neural tube in stage 16 −18 chicken embryos can be observed three somite lengths rostral to the last-formed somite (Bronner-Fraser, 1986). Although HNK-1-positive neural crest cells emerge in an unsegmented pattern along the entire dorsal surface of the neural tube, they only enter the rostral half of each somite. The first neural crest cells enter the somites at the time of sclerotome formation. In embryos double-labelled with anti-T-cadherin and HNK-1 antibodies, we observed neural crest cells entering the rostralmost portion of the somite slightly after or concomitant with the appearance of T-cadherin in the caudalmost portion of the same somite.

The number of neural crest cells progressively increased with distance from the last somite. Relatively few neural crest cells were detected in the ten caudalmost somites. Those were widely dispersed throughout the rostral half of each sclerotome and had no clear orientation. The distribution of the HNK-1 neural crest cells was complementary to the diffuse staining for T-cadherin in the caudal halves of the same somites (Fig. 3B).

In more mature regions of the embryo (11 or more segments above the most recently formed somite), the somites appeared more compact. Larger numbers of neural crest cells with a clear orientation in the





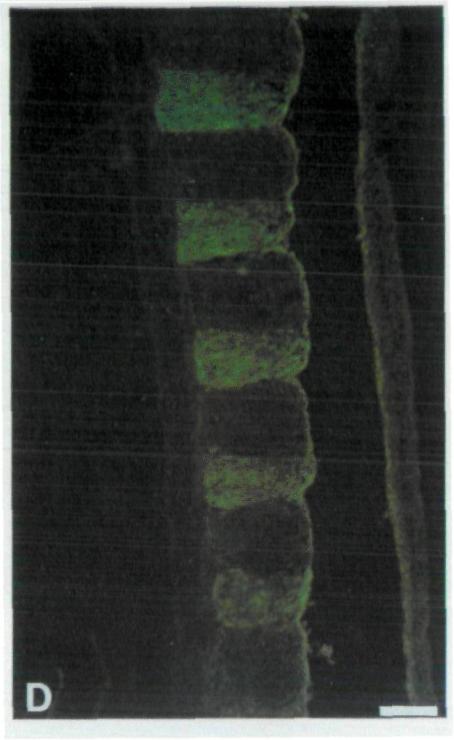
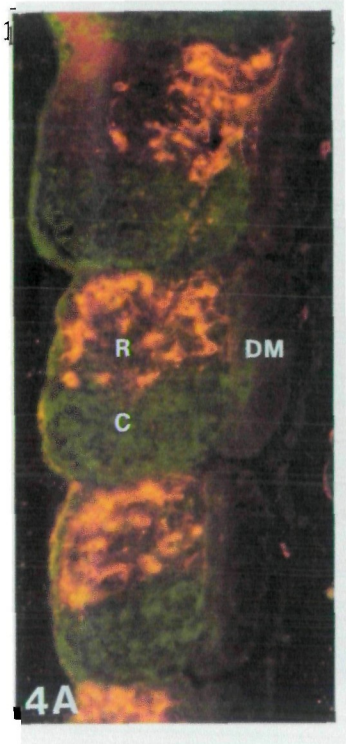
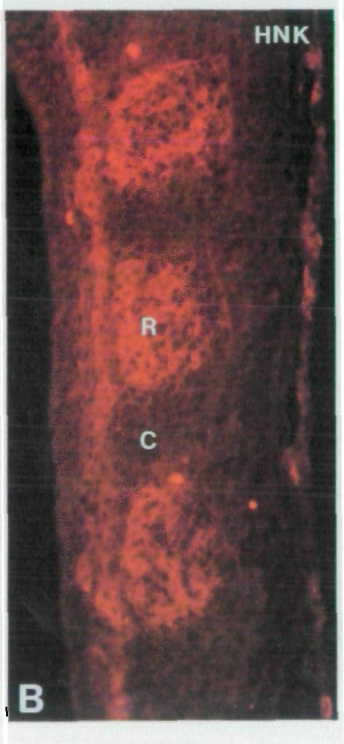
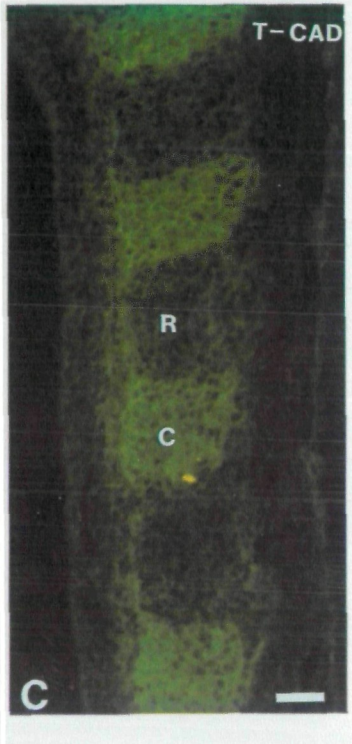


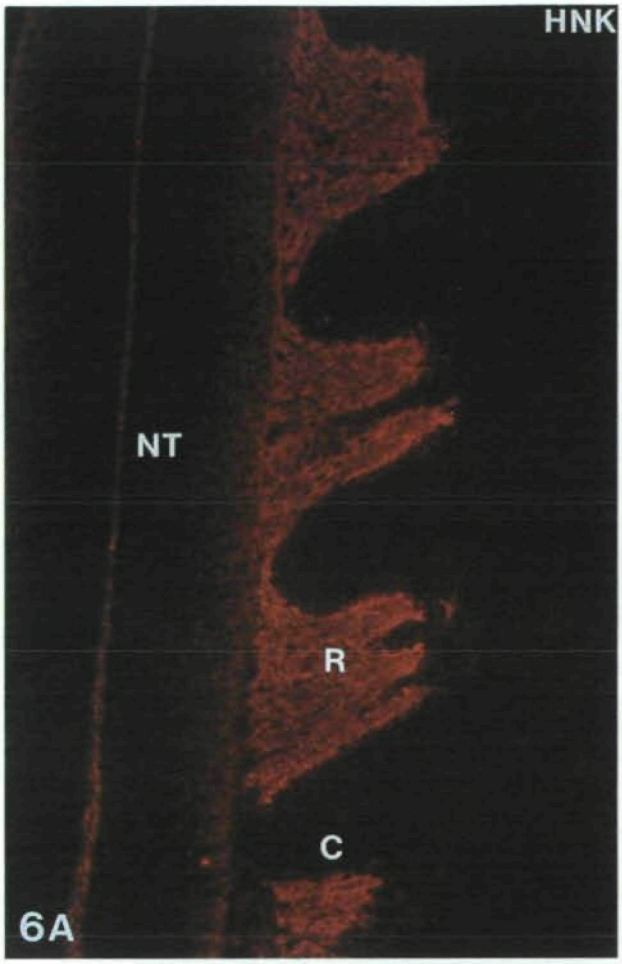
Fig. 3. Fluorescence photomicrographs illustrating the distribution of T-cadherin (A, C, and D) and HNK-1 antigen (B) in longitudinal sections through stage 17 embryos. (A,B) T-cadherin is present in the caudal half of each sclerotome whereas neural crest cells, recognized by the HNK-1 antibody are present in the rostral half. The T-cadherin immunoreactivity is graded along the rostrocaudal axis with more intense staining in more rostral regions. (C) High magnification view of the third somite above the most recently formed somite of the embryo picture in A,B. T-cadherin immunoreactivity appears in a few cells in the caudalmost region of the somite, with more diffuse staining in the caudal sclerotome; neural crest cells in this sections (not shown) are situated between the neural tube and somite and about to enter the rostral portion of the somite. This somite represents the caudal limit of the T-cadherin staining. (D) Another stage 17 embryo illustrating the gradation in T-cadherin staining from more rostral to more caudal levels. Bar=209, 209, 20, and 80 μm in A,D, respectively.

Fig. 4. Fluorescence photomicrographs of sagittal sections through the rostral somites of a stage 17 embryo. (A) Double exposure micrograph of a section illustrating the distribution of neural crest cells (red) and T-cadherin (green), which are inversely correlated. (B,C) Another section showing the distribution of neural crest cells (B) and T-cadherin (C). The neural crest cells are restricted to the rostral half of the sclerotome whereas T-cadherin is in the caudal half of the sclerotome, where it appears associated with the somitic cell surface. R=rostral, C=caudal, DM=dermomyotome. Bar=40 μm.





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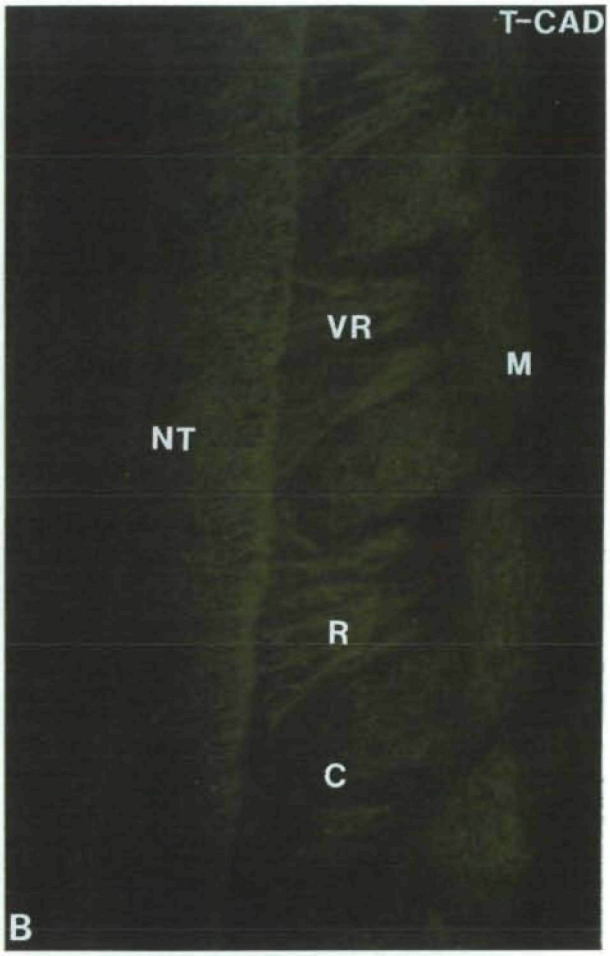
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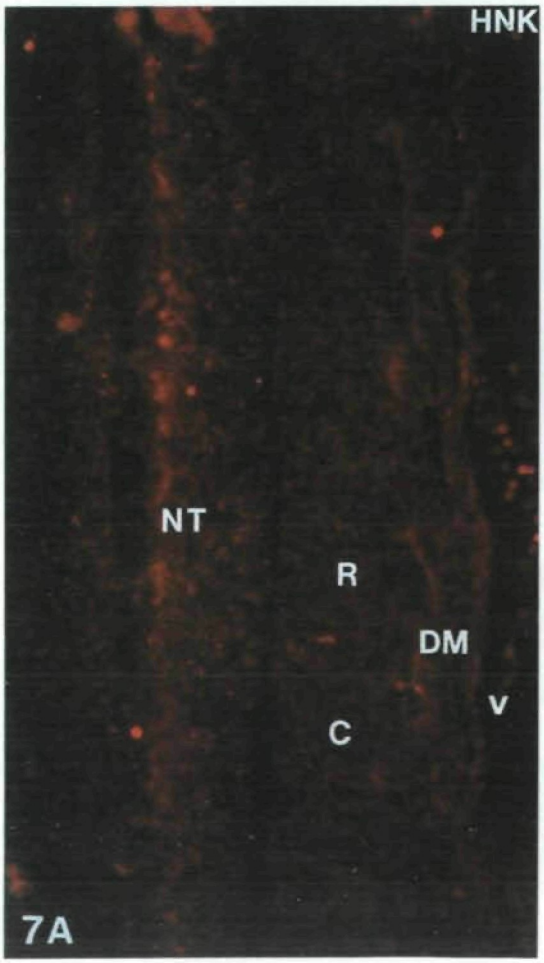
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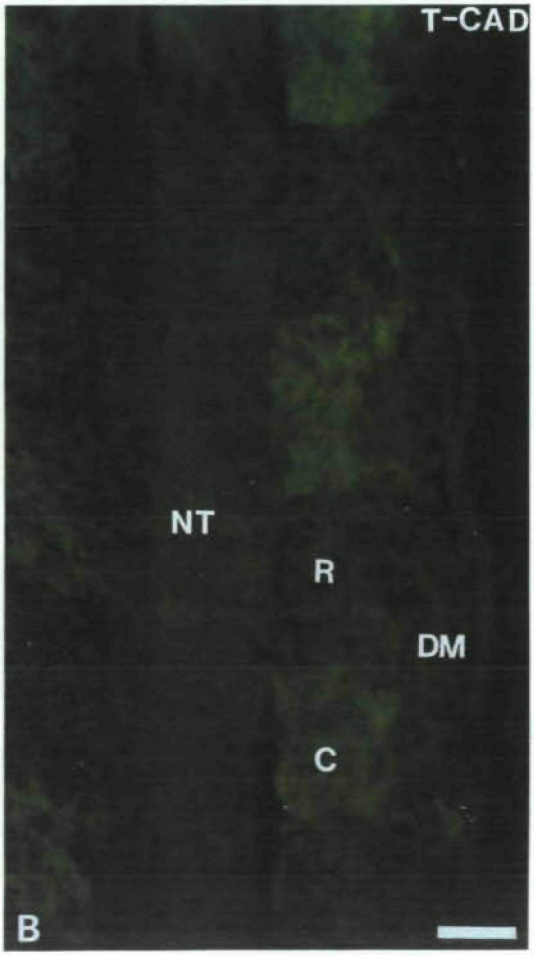
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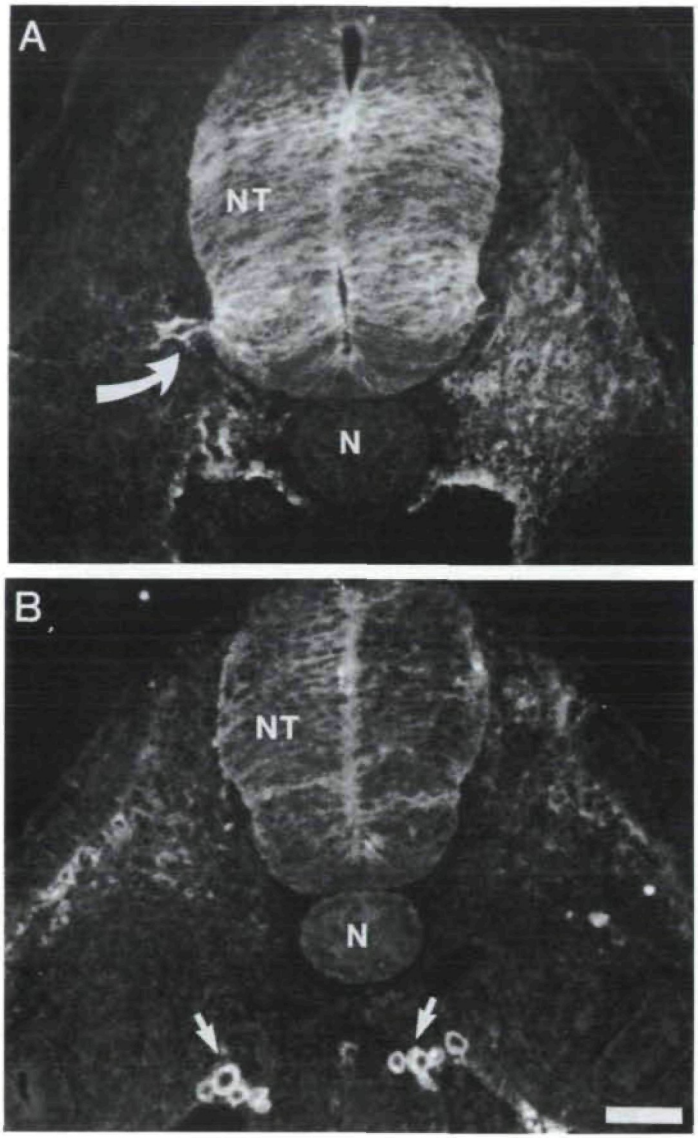

Fig. 5. Fluorescence photomicrographs of transverse sections through the rostral (A) and caudal (B) portions of stage 18 embryos after staining with T-cadherin. (A) A glancing section with the left-hand side through the rostral (R) sclerotome and the right-hand side through the caudal (C) sclerotome. T-cadherin immunoreactivity was prominent on the motoraxons (indicated by curved arrow). Although T-cadherin immunoreactivity was absent from the rostral sclerotome, it was noted throughout the caudal sclerotome, including the region around the notochord (N). (B) In more caudal regions of the embryo, T-cadherin immunoreactivity appeared fibrillar within the caudal sclerotome and was faint or absent in the neural tube. Immunoreactivity was absent from the notochordal region. Intense staining was observed on primordial germ cells (indicated by straight arrows). Bar=47 μm.

mediolateral direction were migrating through the rostral half of each sclerotome. At these more mature axial levels, the expression of T-cadherin was more pronounced than in more caudal regions of the embryo. The boundary between the T-cadherin-positive caudal halves and T-cadherin-negative rostral halves of the sclerotomes was sharp, with T-cadherin expression alternating with the HNK-1-positive neural crest cells (Fig. 4).

T-cadherin expression in stage 19 embryos

By stage 19, neural crest cells along the ventral pathway are in their final stages of migration and are beginning to aggregate to form dorsal root and sympathetic ganglia. At this time (Fig. 6), T-cadherin immunoreactivity remained prominent in the caudal half of the sclerotome, but now was also observed throughout the myotome, though it was absent from the dermomyotome at earlier stages. It contrast to the sclerotome, staining of the myotome was non-segmented. T-cadherin was detected on motor axons that, like neural crest cells, move through the rostral half of each sclerotome.

Cell density in the somites

One possible explanation for the absence of neural crest cells from the caudal half of the sclerotome is that close cell packing in this region may mechanically exclude neural crest cells. To investigate if T-cadherin expression correlates with a possible compaction of the caudal-half sclerotomal cells, we compared the length of the somites and the density of sclerotomal cells in the rostral *versus* caudal halves of the sclerotome. The number of nuclei in a 1200 μm^2 area was determined in randomly chosen sections through the rostral and caudal halves of the sclerotome at 5, 7, 9, 13, and 15 somites above the last-formed somite in stage 16–17 embryos. Nuclei of HNK-1-positive neural crest cells were substracted prior to quantitation. The presence or absence of HNK-1 immunoreactivity also distinguished the rostral half (neural crest-containing) from the caudal half (neural crest-free) of the sclerotome. The cell density in the caudal half of the sclerotome was 43–67 % higher than that in the rostral half at all somite levels examined ($P \leq 0.001$; Table 1). Therefore, the cell density in the caudal half of the sclerotome increases

Fig. 6. Fluorescent photomicrographs of longitudinal sections through a stage 19 embryo. (A) At the level of the ventral neural tube, neural crest-derived Schwann cells and motoraxons stain with the HNK-1 antibody. (B) In the same section, T-cadherin immunoreactivity is observed on the motoraxons in the rostral (R) half of the sclerotome as well as in the caudal (C) half sclerotomal cells, myotomal (M) cells and cells in the ventral neural tube (NT). Bar=80 μm.

Fig. 7. Fluorescent photomicrographs of two embryos fixed at stage 17 from which the dorsal neural tube was removed by surgical ablation at stage 11–12. Longitudinal sections are illustrated at the level of the neural tube (NT). Even in the absence of neural crest cells assessed by the lack of HNK-1 immunoreactivity in the rostral half of the sclerotome, T-cadherin immunoreactivity is observed in the caudal half of the sclerotome (B), where it appears on schedule. R=rostral; C=caudal; DM=dermomyotome. Bar=40 μm.

Table 1. *Somite length and sclerotomal cell density in stage 16–17 embryos*

	Rostrocaudal extent (μm)	Number of cells±S.D./ 1200 μm^2		Ratio caudal/ rostral
		Rostral	Caudal	
Somite 5 (n=19)	218±25	9.4±2.8*	14.6±2.7	1.55
Somite 7 (n=18)	218±20	10.5±3.7*	16.3±2.8	1.55
Somite 9 (n=20)	221±34	10.3±2.5*	15.8±4.1	1.53
Somite 13 (n=8)	198±23	10.4±2.2*	17.4±2.4	1.67
Somite 15 (n=14)	205±34	11.5±2.6*	16.4±3.7	1.43

Somites are numbered with respect to the most recently formed somite.

*Significant differences between cell density in rostral and caudal halves of the sclerotome; $P \leq 0.001$.

shortly after the initial expression of T-cadherin, and this increased density persists with somitic maturation.

T-cadherin expression is independent of signals from neural crest cells

Surgical removal of the dorsal portion of the neural tube in the trunk region produces embryos lacking the neural crest in the operated region (Yntema and Hammond, 1945). To determine if T-cadherin expression depends on the presence of neural crest cells, the neural crest was ablated by surgically removing either the whole neural tube (n=3) or the dorsal portion of the neural tube (n=13). Fifteen embryos completely lacked neural crest cells over several somite lengths either unilaterally (n=3) or bilaterally (n=12), with one additional embryo having a greatly reduced neural crest cell population in the ablated region. In most operated animals, neural crest cells were present rostral and caudal to the ablated region.

In the absence of HNK-1-positive neural crest cells, T-cadherin immunoreactivity persisted in the caudal half of the sclerotome. The staining pattern appeared identical to that observed in unoperated embryos (Fig. 7). The caudalmost extent of immunostaining was detected about three segments rostral to the last-formed somite and immunoreactivity was confined to the caudal half of each sclerotome, increasing in intensity in progressively more mature somites. T-cadherin staining was similar to that of normal animals in longitudinal sections both at the level of the neural tube (Fig. 7A,B) and the notochord (not shown). These observations indicate that the segmental expression of T-cadherin is independent of the presence of neural crest cells.

Discussion

T-cadherin is a novel member of the cadherin cell adhesion family that shares significant similarities with known cadherins in its biochemical characteristics and extracellular structure (Ranscht and Dours-Zimmermann, 1991). T-cadherin, however, differs from known cadherins in that it lacks cytoplasmic sequences that are highly conserved in other cadherins. The polyclonal anti-T-cadherin antiserum used in this study recognizes a predominant polypeptide of 90×10^3 M_r on immunoblots of somites. This pattern compares well with that observed in other tissues (Ranscht and Dours-Zimmerman, 1991) and suggests that genuine T-cadherin is detected in somites.

Here, we report the selective distribution of T-cadherin in the caudal half of each sclerotome during neural crest cell migration as examined by immunohistochemistry. The segmental expression of T-cadherin was inversely correlated with the pattern of migrating neural crest cells at all times examined. T-cadherin immunoreactivity appeared graded along the rostrocaudal axis, with the staining intensity in the caudal half of the sclerotome increasing with distance from the most recently formed somite. T-cadherin immunoreactivity first appeared on a small population of cells in the caudal portion of the somite approximately three somites rostral to the most recently formed somite. Its expression was concurrent with the initial entry of neural crest cells into the rostralmost portion of the same somite. Several cell diameters separate the entry point of neural crest cells into the rostral half of the somite from cells expressing T-cadherin in the caudal half of the somite. This would argue against a direct contact-mediated repulsion or mechanical exclusion mechanism of T-cadherin in the initial selection of the rostral half of the sclerotome by neural crest cells. It is possible, however, that T-cadherin is expressed in a caudal-to-rostral gradient along the entire newly formed somite, despite the fact that such a subtle gradient cannot be detected by immunocytochemistry. The possibility that T-cadherin acts as a diffusible molecule cannot be ruled out since it lacks amino acids conserved in the cytoplasmic domains of other cadherins (Ranscht and Dours-Zimmerman, 1991).

As somites undergo the transition to form the dermomyotome and sclerotome, T-cadherin was expressed preferentially in the caudal half of each sclerotome. Staining became increasingly pronounced with sclerotomal maturation and, eleven or more somites rostral to the last-formed somite, a sharp discontinuity demarcated the T-cadherin immunostaining in the caudal half of each sclerotome from non-stained rostral-half sclerotomal tissue. Above this level, the somitic sclerotome appeared shortened in rostrocaudal extent, with a significantly greater cell density in the caudal half of the sclerotome than in the rostral half of the sclerotome.

While a number of different mechanisms could explain the segmental organization of the peripheral nervous system in vertebrates (Keynes and Stern, 1988), convincing evidence has been presented that the mechanisms responsible for segmentation reside within the somites themselves (Keynes and Stern, 1984; Stern and Keynes, 1987; Oakley and Tosney, 1989; Bronner-Fraser and Stern, 1991). Molecules expressed in either half of the somitic sclerotome are possible candidates for influencing or determining segmentation in vertebrates. Neural crest cells may preferentially interact

with molecules in the rostral somite region, while molecules expressed in the caudal somites may be less permissive or inhibitory for neural crest cell migration. The selective interaction of neural crest cells with cell adhesion molecules in either half of the somitic sclerotome is a compelling possibility to explain their metameric migration pattern. An alternative or additional possibility is that cell adhesion molecules selectively expressed by caudal sclerotomal cells cause cell compaction and mechanical exclusion of cells and/or axons from this area. Consistent with the latter possibility, we observed significantly higher cell densities, apparently correlating with increased levels of T-cadherin expression, in caudal-half sclerotomes compared to their rostral counterparts. This observation, however, does not rule out the possibility that T-cadherin also may act as a less permissive or inhibitory substrate for neural crest cells than molecules expressed in the rostral sclerotome.

The expression of T-cadherin during somite development is distinct in several aspects from the staining pattern described by others for N-cadherin (Hatta *et al.* 1987; Duband *et al.* 1988). Unlike T-cadherin, which was not detected in the segmental plate or in the most recently formed epithelial somites, N-cadherin is detected in the segmental plate and the epithelial somite, predominantly on the apical cell surfaces lining the somitic lumen (Duband *et al.* 1988). At the time of sclerotome formation, T-cadherin was detected on a small population of cells in the caudal portion of the somite. In more posterior regions of the embryo, there may be some overlap between T-cadherin and N-cadherin in the caudal portion of the somite. As the epithelial somites form the dermomyotome and sclerotome, T-cadherin expression persisted specifically in the caudal sclerotome, while N-cadherin seems to disappear completely from sclerotomal cells. The transition from N-cadherin to T-cadherin expression during sclerotome formation may reflect the epithelial–mesenchymal transition, consistent with the proposed role of cadherins in tissue morphogenesis (Takeichi, 1988).

To date, only one group of molecules other than T-cadherin has been localized specifically to the caudal half of the sclerotome during neural crest migration and motor axon growth. Stern *et al.* (1986) and Davies *et al.* (1990) described peanut agglutinin (PNA) binding glycoproteins of 48 and 55×10^3 M_r that are expressed in the caudal half of the sclerotome. These molecule(s) differ from T-cadherin not only in their apparent molecular mass, but also in their glycosylation and pattern of expression. T-cadherin has an apparent relative molecular mass of 90×10^3 M_r and exhibits no apparent peanut lectin binding activity in stage 16–17 somite homogenates (Ranscht, unpublished observation). In addition, T-cadherin is expressed on motor axons whereas the PNA-binding molecules are not. The PNA-binding proteins cause growth cones to collapse suggesting that inhibitory mechanisms may participate in establishing segmentation in vertebrate embryos (Davies *et al.* 1990). It remains to be established if T-cadherin has a similar function. The anti-T-cadherin antiserum used in this study appears to recognize T-cadherin mainly in a denatured or fixed configuration and was not effective in preliminary functional studies. Because multiple molecules are present in the rostral and caudal halves of the sclerotome (Stern *et al.* 1986; Norris *et al.* 1989; Layer *et al.* 1988; Tanaka and Obata, 1989; Davies *et al.* 1990), it may be a mistake to argue for a causal role for only one molecule; instead, a combination of cell adhesion molecules, extracellular matrix components, and soluble factors may be required for the establishment and maintenance of somite polarity.

For several reasons, T-cadherin is a logical candidate to play a central role in maintaining rostrocaudal polarity in the somites and to contribute to the metameric migration of neural crest cells. First, T-cadherin expression is confined primarily to the caudal half of the sclerotome as early as the sclerotome is formed and is, therefore, one of the first obvious manifestations of somite polarity. Second, T-cadherin is expressed before or concomitant with neural crest cell immigration into the sclerotome. Third, T-cadherin localization is maintained in a polarized fashion both during neural crest migration and motor axon growth. Fourth, high levels of T-cadherin expression correlate with close packing of cells in the caudal half of the sclerotome. One possible mode of operation is that T-cadherin causes dense packing of caudal-half sclerotomal cells, thereby mechanically excluding neural crest cells and possibly axons from these regions. Alternatively, and perhaps in addition, T-cadherin may function similarly to the PNA-binding proteins (Davies *et al.* 1990) by acting as an inhibitory or repulsive substrate for neural crest cell and/or growth cone locomotion. Although functional tests will be required to establish the role of T-cadherin in development, its spatial and temporal distribution and homology to cadherins make it an attractive candidate for influencing the pattern of spinal segmentation in vertebrate embryos.

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